Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 40-43, 45-56 and 58-60 are pending in the application, with claim 40 being the independent claim. Applicants have amended claim 40 in order to clarify the claimed invention. These changes are believed to introduce no new matter and are believed to place the application in condition for allowance. Thus, entry of the foregoing amendment is respectfully requested.

Based on the above Amendment and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

I. Amendment of Claim 40

The Examiner has indicated that claim 40 contains an intended use limitation which does not carry patentable weight. Office Action, Page 3. Applicants note that the claimed invention is directed to a DNA molecule comprising (1) DNA encoding the OmpA signal peptide; (2) DNA encoding a tPA or K2S molecule; and (3) DNA encoding either the SEGN or SEGNSD peptide, where the extracellular secretion attribute of the DNA molecule is an *inherent* feature of the molecule and not an intended use limitation. In order to clarify the nature of the invention and to emphasize that the extracellular secretion attribute is an inherent property of the claimed DNA molecule, Applicants have amended claim 40 so that it no longer recites the phrase "wherein a prokaryotic host cell transformed with said DNA molecule secretes said tPA or K2S molecule

extracellularly as a thrombolytically active protein." As described in detail below, the claimed DNA molecule is novel and nonobvious in view of its *inherent* ability to be expressed and secreted by a prokaryotic host cell extracellularly as a thromoblytically active protein.

II. The Examiner has not established a prima facie case of obviousness

The Examiner has rejected claims 40-56 and 58-60 under 35 U.S.C. § 103 as allegedly being obvious over Wang *et al.* (EP 0357391 A2) (IDS document AM1) [hereinafter "Wang"] in view of Obukowicz *et al.*, *Biochemistry 29*:9737-9745 (1990) (IDS document AR9) [hereinafter "Obukowicz"], Niwa *et al.* (U.S. Patent No. 5,840,533) [hereinafter "the '533 patent"] and the nucleic acid encoding human tissue plasminogen activator, for the reasons set forth in the prior Office Actions mailed September 12, 2003 and May 4, 2004. Office Action, Page 2.

The Examiner has stated that none of the references teach the claimed invention, but has alleged that he has met his burden in establishing a *prima facie* case of obviousness against the claims. Office Action, Page 3. Applicants respectfully disagree and traverse the rejection.

In order to establish a *prima facie* case of obviousness, the following three criteria must be met: (1) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) there must be a reasonable expectation of success; and (3) the prior art reference (or references when combined) must teach or suggest all the claim limitations. MPEP § 2143. Furthermore,

without a motivation to combine, a rejection based on a *prima facie* case of obviousness is improper. *See In re Rouffet*, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1457-58 (Fed. Cir. 1998).

Applicants assert that there is no suggestion or motivation to combine the reference teachings and thus the first criteria necessary to establish a *prima facie* case of obviousness has not been met. Applicants again note that the claimed invention is directed to a DNA molecule comprising: (1) tPA or K2S; (2) OmpA *and*; (3) either the signal peptide sequence SEGN or SEGNSD. Applicants emphasize that the combination of using *both* OmpA and SEGN/SEGNSD in the claimed invention results in the secretion of tPA or K2S into the culture medium, where the secreted tPA or K2S is properly folded and thrombolytically active. Furthermore, it is the combination of using *both* OmpA and SEGN/SEGNSD which allows for the recovery of a larger than expected fraction of the tPA or K2S to be secreted into the culture supernatant of *E. coli*. This in turn allows for a cost effective means for large scale production of biologically active tPA or K2S.

The references cited by the Examiner do not suggest or provide motivation for the inclusion of a SEGN or SEGNSD peptide within a fusion construct which also contains OmpA and tPA or K2S.

The Examiner states that the '533 patent discloses SEQ ID NO:1, corresponding to a K2S sequence which also includes either SEGN or SEGNSD at its N-terminus. Office Action, Page 3. In addition, the Examiner also alleges that the '533 patent discusses signal peptide without specific reference to any particular cell or expression system. *Id.*

Applicants note that the '533 patent does not disclose the OmpA signal sequence. While the '533 patent discusses the use of a signal peptide, the only embodiment disclosed is the signal peptide of tPA. The '533 patent, col. 5, ll. 12-13. The '533 patent does not discuss the use of other specific signal peptides, such as OmpA, to be used in a fusion construct together with the identified tPA variant.

In addition, the '533 patent provides no motivation or suggestion to modify SEQ ID NO:1 in order to optimize secretion of this sequence into the culture supernatant after expression in *E. coli*. With respect to expression in *E. coli*, the '533 patent only discusses the purification of proteins from cell debris. Specifically, the '533 patent states:

> [w]hen a bacterium such as *E. coli* is used as a host cell, thus produced new t-PA generally exist in cells of the cultured transformant and the cells are collected by filtration or centrifugation, and cell wall and/or cell membrane thereof are destroyed in a conventional manner . . . to give debris. From the debris, the new t-PA can be purified and isolated . . .

The '533 patent, col. 6, lines 21-27.

Therefore, a person skilled in the art who wishes to improve the secretion properties of tPA or K2S in bacteria, would not get any information, idea or motivation to combine the OmpA-leader with the SEGN or SEGNSD peptides in order to achieve secretion of K2S variants into the culture supernatant when expressed in *E. coli*.

Moreover, the '533 patent is directed to characterizing a new variant of tPA which has a longer half-life and an increased thrombolytic activity. The '533 patent, Abstract; col. 2, ll. 13-19. In view of this, a person skilled in the art would not utilize the

teaching of the '533 to determine, for example, what constructs may allow secretion of K2S variants into the culture supernatant when expressed in *E. coli*.

In summary, the '533 patent describes a tPA variant with increased thromobolytic activity, but does not suggest or provide any motivation to use an OmpA signal peptide which would allow or facilitate the secretion of tPA or tPA variants into the culture supernatant when expressed in *E. coli*. Thus, one of ordinary skill in the art, considering the disclosure of the '533 patent, would not have been motivated to generate a construct which contains the OmpA signal sequence, the amino acid sequence SEGN or SEGNSD and tPA or K2S.

The Examiner also discusses Wang, which discloses an expression system in *E. coli* where a *heterologous* protein is fused in frame to a nucleic acid sequence encoding the OmpA signal peptide. Office Action, Page 3. Wang does not disclose the amino acid sequence SEGN or SEGNSD. In addition, Wang does not provide any motivation to include SEGN or SEGNSD within a fusion construct containing OmpA and a heterologous protein. Examples of proteins disclosed in Wang include parathyroid hormone (PTH), epidermal growth factor (EGF), interleukin-6 (IL-6), and CD4. Wang, Example 2. Wang, however, does not contemplate using OmpA to secrete a protein, such as tPA. As shown in the art, the properties of tPA, such as its disulfide bonds, provide unique challenges which make it difficult to secrete tPA using OmpA alone. *See e.g.*, U.S. Patent No. 6,027,888, col. 4, lines 12-18. Wang does not consider this problem, and thus provides no motivation to combine its teaching with one which discloses the use of SEGN/SEGNSD. Thus, one of ordinary skill in the art, considering

the Wang reference, would not be motivated to combine this teaching with, for example, the '533 patent reference discussed above.

Obukowicz teaches expression of a fusion construct comprising the signal peptide PhoA and the K2S protein. Obukowicz, however, does not teach or suggest a fusion construct comprising a sequence encoding the peptide SEGN or SEGNSD.

Additionally, Obukowicz notes the unpredictability of secreting the K2S sequence (referred to as MB1004 in their study) and further points out that attempts to secrete tPA using other Omp family signal peptides have failed. Specifically, Obukowicz states:

> The success with MB1004 secretion using the *phoA* leader sequence in combination with the *phoA* ribosome binding site and *tac* promoter illustrates the unpredictability of whether secretion will occur. Previous attempts to secret MB1004 or just the serine protease domain of tPA using the *lamB* or *ompF* leader sequence in combination with the phage T7 g10L ribosome binding site and *recA* promoter failed. The failure in secretion of full-length tPA using the *ompA* or native tPA leader sequence has also been reported (Sarmientos et al., 1989).

Obukowicz, Page 9744.

Thus, Obukowicz *teaches away* from combining the OmpA signal sequence with a K2S sequence, as their own study using other Omp signal sequence shows the resulting failure in doing so.

The sequence encoding human tissue plasminogen activator does not rescue the deficiencies of the '533 patent, Wang, or Obukowicz as it merely teaches the sequence of

human tPA, and does not provide any suggestion for a nucleic acid sequence encoding tPA to be operably linked to the signal sequence OmpA or the sequence encoding either the peptide SEGN or SEGNSD.

The mere fact that a reference can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990). As shown above, none of the references described above provide motivation or suggest the desirability of the combination of OmpA, SEGN/SEGNSD, and tPA within an expression construct. Thus, Applicants assert that the criteria requiring that a suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings, has not been met. Therefore, a *prima facie* case of obviousness, with respect to claims 40-56 and 58-60, has not been established. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 103 be reconsidered and withdrawn.

III. Even assuming that a prima facie case of obviousness has been established, Applicants assert that this prima facie case of obviousness can be rebutted

Assuming, *arguendo*, that the Examiner has established a *prima facie* case of obviousness, Applicants assert that the *prima facie* case of obviousness can be rebutted. Rebuttal evidence may include evidence of "secondary considerations," such as "commercial success, long felt but unsolved needs, [and] failure of others." *Graham v. John Deere Co.*, 383 US at 17, 148 USPQ at 467. Rebuttal evidence may also consist of a showing that the claimed compound possesses unexpected properties. *Dillon*, 919 F. 2d at 692-93, 16 USPQ2d at 1901.

1. Long-felt need in the art

The prior art provides numerous examples of the long-felt need to provide an expression technology which allows secretion and purification of tPA or K2S variants, correctly folded and in an active manner, from the culture supernatant. For example, U.S. Patent No. 6,027,888 [hereinafter "the '888 patent"] states:

> Therefore what is lacking in the prior art are methods, recombinant vectors host cells, and compositions comprising high-level expression of eukaryotic disulfide bond containing polypeptides (such as tPA and BPTI) which are soluble, correctly folded, active and readily isolatable from cell extracts of prokaryotic hosts.

The '888 Patent, col. 4, lines 12-18.

The '888 patent describes an expression system which allows expression and purification of tPA from the periplasmic space. The '888 patent, col. 13, l. 30 to col. 15, l. 61.

The disadvantages of a system which involve purification of proteins from the periplasmic space are described in the art. For example, EP 1 048 732 (IDS document AL2) states:

> When recombinant DNA is expressed in prokaryotic host organisms, it is often desirable to secrete the recombinant gene product or protein that is obtained in this process from the cytoplasm through the inner bacterial membrane into the periplasmic space between the inner and outer membrane. Secreted proteins can then be released from the periplasm into the nutrient medium for example by osmotic shock. A disadvantage

> of this process is that the secreted polypeptides often do not form the native, biologically active conformation.

EP 1 048 732, page 2, lines 5-10.

In contrast, the prior art discusses the advantages of a system where protein can be secreted and purified from the culture medium of prokaryotic host cells. For example, EP 357 391 (IDS document AM1) states:

> To facilitate protein recovery process it is preferable that protein accumulates in the culture medium during culturing of the protein-producing *E coli* host, since there are relatively fewer contaminants and the protein can be recovered without damaging its cellular source. There have been various attempts at developing such a system, for extracting protein to the medium, all of which include some tactic for overcoming the integrity of the outer membrane barrier.

EP 357 391, page 2, lines 22-27.

Additional approaches in the art used to overcome the problem of producing a soluble enzymatically active form of tPA or tPA variants in bacteria substantiate the long-felt need in the art to provide an expression technology which allows secretion of tPA or K2S variants, correctly folded and in an active manner, into the culture supernatant, from which they can be easily purified. In particular, EP 302 456 to Niwa *et al.* (IDS document AL1) modify the coding sequence of tPA in order to obtain a soluble, enzymatically active form of tPA or a variant thereof in *E. coli*. The study of EP 1 077 263 to Schreiner *et al.* (IDS document AM2) attempts to improve bacterial metabolism

by co-expressing proteins involved in the protein folding machinery in order to obtain a properly folded active protein.

2. Failure of others

Various studies in the prior art attempting to secrete and purify proteins from the culture medium of prokaryotic hosts have failed with regard to the expression of tPA and K2S.

Sarmientos *et al.*, *Bio/Technology* 7: 495-499 (1989) [hereinafter "Sarmientos"] (IDS document AT10), investigated whether a bacterial expression system could be used to recover active tPA from *E. coli* at a significant level. Sarmientos, 495. In their study, Sarmientos demonstrated that neither the human tPA leader sequence, nor the OmpA leader peptide functioned as efficient secretion signal sequences in *E. coli*. Sarmientos, 497. In particular, they stated that "[i]n all cases, tPA was expressed as an insoluble protein and preliminary evidence indicated that the resulting protein migrated slightly differently from mature tPA on PAGE-SDS, further suggesting that secretion into the *E. coli* periplasm could not be used for efficient production of matured and active tPA." *Id.*

Hua *et al.*, *Science in China*, pp. 667 - 676 (1994) [hereinafter "Hua"] (IDS document AT13) describe the synthesis and expression of the kringle-2 domain of tPA in *E. coli* using the OmpA2 signal sequence. Hua, Abstract. In their study, Hua reported that the kringle-2 domain accumulated in the periplasmic space. Hua, Page 675; Abstract.

Cleary *et al.*, *Biochemistry* pp.1884-1891 (1989) [hereinafter "Cleary"] (IDS document AS13) also discuss the purification and characterization of the kringle-2

domain of tPA in *E. coli*, but also report accumulation in the periplasmic space. Cleary, Page 1889; Abstract.

The study of Waldenström *et al.*, *Gene*, 1999, pp. 243 - 248 [hereinafter " Waldenström"] (IDS document AR12) also addresses the problem of purifying and secreting tPA after expression in *E. coli*. In their study, Waldenström found that the production of their fusion protein which contained a fragment of full-length tPA was poor in the *E. coli* strain used. Waldenström, Page 245. Waldenström stated that this was due in part to a large degree of proteolytic degradation, but also hypothesized that this could be due to the high content of rare Arg codons within the tPA sequence. *Id.*

The studies described above show that attempts at generating expression technology which would allow secretion and purification of correctly folded and thrombolytically active tPA or K2S variants from the culture supernatant of prokaryotic cells were largely unsuccessful.

3. Unexpected results

Evidence of unobvious or unexpected advantageous properties, such as superiority in a property the claimed compound shares with the prior art, can rebut *prima facie* obviousness. MPEP § 716.02; *See In re Chupp*, 816 F.2d 643, 646, 2 USPQ2d 1437, 1439 (Fed. Cir. 1987). In the present case, in view of the long-felt need in the art to secrete and purify active tPA from the culture medium of prokaryotic hosts, the finding in the present application that a construct comprising tPA or K2S, OmpA and SEGN or SEGNSD can be secreted and purified as an active protein from the culture medium of *E. coli* is an unexpected result.

The present application provides that when recombinant tPA and/or K2S molecules are expressed in a prokaryotic host cell, they are efficiently secreted into the culture supernatant utilizing the beneficial combination of *both* an OmpA signal peptide and the SEGN or SEGNSD peptide. The specification, at paragraph [0103] notes that when recombinant K2S containing the SEGNSD sequence is expressed in *E. coli* cells, 68% of the recombinant K2S protein can be directly isolated from the culture supernatant, with only 32% of the recombinant K2S secreted into the periplasm. In view of the art discussed above, this level of secretion of a properly folded and enzymatically active tPA or K2S protein into the culture medium, is unexpected.

4. Summary

Applicants assert that even assuming a *prima facie* case of obviousness has been established, a *prima facie* case of obviousness can be rebutted using the evidence described above.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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